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☐ 1: *Plant J* 1995 May;7(5):771-84

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Co-expression of native and introduced genes reveals cryptic regulation of HMG CoA reductase expression in Arabidopsis.

Re EB, Jones D, Learned RM

Section of Molecular and Cellular Biology, University of California, Davis 95616, USA.

In eukaryotes, all isoprenoid compounds share a common precursor, mevalonic acid, whose synthesis is catalyzed by the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase. As one step towards a better understanding of the role that this enzyme plays in coordinating isoprenoid biosynthesis in plants, *Arabidopsis thaliana* HMG CoA reductase was ectopically expressed in transgenic *Arabidopsis* plants. By using this molecular genetic approach, several novel and fundamental observations have been made regarding isoprenoid biosynthesis in *Arabidopsis*. First, it was demonstrated that the overexpression of authentic *Arabidopsis* HMG CoA reductase is not sufficient to alter the bulk synthesis and accumulation of the abundant end products of the plant isoprenoid pathway. Second, active transcription of the transgene appears to co-activate and deregulate expression of the native gene, resulting in a striking elevation of HMG CoA reductase mRNA levels. Finally, although very high levels of HMG CoA reductase mRNA were expressed in these transgenic plants, only modest increases in enzyme activity could be detected. Taken together, these data suggest that HMG CoA reductase expression is regulated at multiple levels in plants as well as animals, and they provide a foundation for elucidating the molecular mechanisms for mevalonate regulation in *A. thaliana*.

PMID: 7773308, UI: 95291337

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☐ 1: *Science* 1991 May 3;252(5006):675-9

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Modular organization of genes required for complex polyketide biosynthesis.

Donadio S, Staver MJ, McAlpine JB, Swanson SJ, Katz L

Department of Corporate Molecular Biology, Abbott Laboratories, Abbott Park, IL 60064.

In *Saccharopolyspora erythraea*, the genes that govern synthesis of the polyketide portion of the macrolide antibiotic erythromycin are organized in six repeated units that encode fatty acid synthase (FAS)-like activities. Each repeated unit is designated a module, and two modules are contained in a single open reading frame. A model for the synthesis of this complex polyketide is proposed, where each module encodes a functional synthase unit and each synthase unit participates specifically in one of the six FAS-like elongation steps required for formation of the polyketide. In addition, genetic organization and biochemical order of events appear to be colinear. Evidence for the model is provided by construction of a selected mutant and by isolation of a polyketide of predicted structure.

PMID: 2024119, UI: 91220065

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☐ 1: *J Bacteriol* 1995
Nov;177(22):6575-84

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**Structure and functional analysis of a marine bacterial
carotenoid biosynthesis gene cluster and astaxanthin
biosynthetic pathway proposed at the gene level.**

Misawa N, Satomi Y, Kondo K, Yokoyama A, Kajiwarra S, Saito T, Ohtani
T, Miki W

Central Laboratories for Key Technology, Kirin Brewery Co., Ltd., Kanagawa,
Japan.

A carotenoid biosynthesis gene cluster for the production of astaxanthin was isolated from the marine bacterium *Agrobacterium aurantiacum*. This cluster contained five carotenogenic genes with the same orientation, which were designated crtW, crtZ, crtY, crtI, and crtB. The stop codons of individual crt genes except for crtB overlapped the start codons of the following crt genes. *Escherichia coli* transformants carrying the *Erwinia uredovora* carotenoid biosynthesis genes provide suitable substrates for carotenoid biosynthesis. The functions of the five crt genes of *A. aurantiacum* were determined through chromatographic and spectroscopic analyses of the pigments accumulated in some *E. coli* transformants carrying various combinations of the *E. uredovora* and *A. aurantiacum* carotenogenic genes. As a result, the astaxanthin biosynthetic pathway is proposed for the first time at the level of the biosynthesis genes. The crtW and crtZ gene products, which mediated the oxygenation reactions from beta-carotene to astaxanthin, were found to have low substrate specificity. This allowed the production of many presumed intermediates of astaxanthin, i.e., adonixanthin, phoenicoxanthin (adonirubin), canthaxanthin, 3'-hydroxyechinenone, and 3-hydroxyechinenone.

PMID: 7592436, UI: 96062243

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☐ 1: *Genomics* 1995 Sep
1;29(1):16-23

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Human estrogen sulfotransferase gene (STE): cloning, structure, and chromosomal localization.

Her C, Aksoy IA, Kimura S, Brandriff BF, Wasmuth JJ, Weinshilboum RM

Department of Pharmacology, Mayo Medical School, Rochester, Minnesota 55905, USA.

NEW

Sulfation is an important pathway in the metabolism of estrogens. We recently cloned a human liver estrogen sulfotransferase (EST) cDNA. We have now determined the structure and chromosomal localization of the EST gene, STE, as a step toward molecular genetic studies of the regulation of EST in humans. STE spans approximately 20 kb and consists of 8 exons, ranging in length from 95 to 181 bp. The locations of most exon-intron splice junctions within STE are identical to those found in a human phenol ST (PST) gene, STM, and in a rat PST gene. In addition, the locations of five STE introns are also conserved in the human dehydroepiandrosterone (DHEA) ST gene, STD. The 5'-flanking region of STE contains one CCAAT and two TATA sequences. The location of one of the TATA box elements is in excellent agreement with the site of transcription initiation as determined by 5'-rapid amplification of cDNA ends. STE was mapped to human chromosome 4q13.1 by fluorescence in situ hybridization. Cloning and structural characterization of STE will now make it possible to study potential molecular genetic mechanisms involved in the regulation of EST in human tissues.

PMID: 8530066, UI: 96079087

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☐ 1: *J Bacteriol* 1993 Apr;175(8):2197-204

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Genetic construction and functional analysis of hybrid polyketide synthases containing heterologous acyl carrier proteins.

Khosla C, McDaniel R, Ebert-Khosla S, Torres R, Sherman DH, Bibb MJ, Hopwood DA

Department of Genetics, John Innes Institute, John Innes Centre, Norwich, United Kingdom.

NEW

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The gene that encodes the acyl carrier protein (ACP) of the actinorhodin polyketide synthase (PKS) of *Streptomyces coelicolor* A3(2) was replaced with homologs from the granaticin, oxytetracycline, tetracenomycin, and putative frenolicin polyketide synthase gene clusters. All of the replacements led to expression of functional synthases, and the recombinants synthesized aromatic polyketides similar in chromatographic properties to actinorhodin or to shunt products produced by mutants defective in the actinorhodin pathway. Some regions within the ACP were also shown to be interchangeable and allow production of a functional hybrid ACP. Structural analysis of the most abundant polyketide product of one of the recombinants by electrospray mass spectrometry suggested that it is identical to mutactin, a previously characterized shunt product of an actVII mutant (deficient in cyclase and dehydrase activities). Quantitative differences in the product profiles of strains that express the various hybrid synthases were observed. These can be explained, at least in part, by differences in ribosome-binding sites upstream of each ACP gene, implying either that the ACP concentration in some strains is rate limiting to overall PKS activity or that the level of ACP expression also influences the expression of another enzyme(s) encoded by a downstream gene(s) in the same operon as the actinorhodin ACP gene. These results reaffirm the idea that construction of hybrid polyketide synthases will be a useful approach for dissecting the molecular basis of the specificity of PKS-catalyzed reactions. However, they also point to the need for reducing the chemical complexity of the approach by minimizing the diversity of polyketide products synthesized in strains that produce recombinant polyketide synthases.

PMID: 8468280, UI: 93224438



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☐ 1: *EMBO J* 1992 Feb;11(2):785-92 Related Articles, Books, Protein, LinkOut

Cloning of the complete biosynthetic gene cluster for an aminonucleoside antibiotic, puromycin, and its regulated expression in heterologous hosts.

Lacalle RA, Tercero JA, Jimenez A

Centro de Biología Molecular (CSIC and UAM), Universidad Autónoma, Madrid, Spain.

Puromycin, produced by *Streptomyces alboniger*, is a member of the large group of aminonucleoside antibiotics. The genes *pac* and *dmpM*, encoding a puromycin N-acetyl transferase and an O-demethyl puromycin O-methyltransferase, respectively, are tightly linked in the DNA of *S. alboniger*. The entire set of genes encoding the puromycin biosynthesis pathway was cloned by screening a gene library from *S. alboniger*, raised in the low copy number cosmid pKC505, with a DNA fragment containing *pac* and *dmpM*. Puromycin was identified by biochemical and physicochemical methods, including ¹H NMR, in the producing transformants. This pathway was located in a single DNA fragment of 15 kb which included the resistance, structural and regulatory genes and was expressed when introduced into two heterologous hosts *Streptomyces lividans* and *Streptomyces griseofuscus*. In addition to *pac* and *dmpM*, two other genes have been identified in the *pur* cluster: *pacHY*, which determines an N-acetylpuromycin hydrolase and *prg1*, whose deduced amino acid sequence is significantly similar to that of *degT*, a *Bacillus stearothermophilus* pleiotropic regulatory gene.

PMID: 1537349, UI: 92164669

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☐ 1: *Gene* 1994 Dec 30;151(1-2):1-10

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Characterization of the *Streptomyces peucetius* ATCC 29050 genes encoding doxorubicin polyketide synthase.

Grimm A, Madduri K, Ali A, Hutchinson CR

Department of Genetics, University of Wisconsin, Madison 53706.

The *dps* genes of *Streptomyces peucetius*, encoding daunorubicin (DNR)-doxorubicin (DXR) polyketide synthase (PKS), are largely within an 8.7-kb region of DNA that has been characterized by Southern analysis, and gene sequencing, mutagenesis and expression experiments. This region contains nine ORFs, many of whose predicted products are homologous to known PKS enzymes. Surprisingly, the gene encoding the DXR PKS acyl carrier protein is not in this region, but is located about 10 kb distant from the position it usually occupies in other gene clusters encoding type-II PKS. An in-frame deletion in the *dpsB* gene, encoding a putative subunit of the DXR PKS, resulted in loss of production of DXR and the known intermediates of its biosynthetic pathway, confirming that this gene and, by implication, the adjacent *dps* genes are required for DXR biosynthesis. This was verified by expression of the *dps* genes in the heterologous host, *Streptomyces lividans*, which resulted in the production of aklanonic acid, an early intermediate of DXR biosynthesis.

PMID: 7828855, UI: 95129838

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☐ 1: *Gene* 1996 Jun 12;172(1):87-91

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Characterization of *Streptomyces argillaceus* genes encoding a polyketide synthase involved in the biosynthesis of the antitumor mithramycin.

Lombo F, Blanco G, Fernandez E, Mendez C, Salas JA

Departamento de Biología Funcional, Instituto Universitario de Biotecnología de Asturias (I.U.B.A-C.S.I.C), Universidad de Oviedo, Spain.

NEW

Mithramycin (Mtm) is an aromatic polyketide which shows antibacterial and antitumor activity. From a chromosomal cosmid library of *Streptomyces argillaceus*, a Mtm producer, a clone (cosAR7) was isolated by homology to the actI/III region of *S. coelicolor* and the strDEM genes of *S. griseus*. From this clone, a 5.3-kb DNA region was sequenced and found to encode six open reading frames (designated as mtmQXPKST1), five of them transcribed in the same direction. The deduced products of five of these genes resembled components of type-II polyketide synthases. The mtm genes would code for an aromatase (mtmQ), a polypeptide of unknown function (mtmX), a beta-ketoacylsynthase (mtmP) and a related 'chain length factor' (mtmK), an acyl carrier protein (mtmS) and a beta-ketoreductase (mtmT1). The involvement of this gene cluster in Mtm biosynthesis was demonstrated by the Mtm non-producing phenotype of mutants generated in two independent insertional inactivation experiments.

PMID: 8654997, UI: 96257259

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☐ 1: *Gene* 1993 Aug 16;130(1):107-16

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Hybridization and DNA sequence analyses suggest an early evolutionary divergence of related biosynthetic gene sets encoding polyketide antibiotics and spore pigments in *Streptomyces* spp.

Blanco G, Brian P, Pereda A, Mendez C, Salas JA, Chater KF

Departamento de Biología Funcional, Universidad de Oviedo, Spain.

NEW

The *whiE* gene cluster of *Streptomyces coelicolor*, which is related to gene sets encoding the biosynthesis of polycyclic aromatic polyketide antibiotics, determines a spore pigment. Southern blotting using probes from three different parts of the *whiE* cluster revealed related gene sets in about half of a collection of diverse *Streptomyces* strains. A 5.2-kb segment of one such cluster, *sch*, previously shown to determine spore pigmentation in *Streptomyces halstedii*, was sequenced. Seven open reading frames (ORFs), two of them incomplete, were found. Six of the ORFs resemble the known part of the *whiE* cluster closely. The derived gene products include a ketosynthase (= condensing enzyme) pair, acyl carrier protein and cyclase, as well as two of unidentified function. The seventh ORF diverges from the main cluster and encodes a protein that resembles a dichlorophenol hydroxylase. Comparison with sequences of related gene sets for the biosynthesis of antibiotics suggests that gene clusters destined to specify pigment production diverged from those destined to specify antibiotics early in the evolution of the *Streptomyces* genus.

PMID: 8344517, UI: 93345807

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